AWARD NUMBER: W81XWH-15-1-0234

TITLE: “Establishment of donor Chimerism Using Allogeneic Bone Marrow with AMP Cell Co-infusion”

PRINCIPAL INVESTIGATOR: Megan Sykes

CONTRACTING ORGANIZATION:

Columbia University Medical Center
New York, NY 10032

REPORT DATE: September 2016

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2016	Annual report	15 Aug 2015 - 14 Aug 2016

4. TITLE AND SUBTITLE
Establishment of donor Chimerism Using Allogeneic Bone Marrow with AMP Cell Co-infusion"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0234

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Megan Sykes, Adam Griesemer, Paula Alonso Guallart, Akaitz Dorronsoro Gonzalez, Hao Wei Li

E-Mail: Megan.sykes@columbia.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK
630 W 168th ST FL4, NEW YORK,
NY 10032-3275

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Composite tissue allografts (CTA) have been more and more frequently used to treat combat-associated injuries, however, rejection remains to be the major barrier that could not be solved by potent non-specific immunosuppression. Induction of tolerance to the CTA is the ideal solution. Combined mixed allogeneic chimerism induction and kidney transplantation has been shown to induce robust tolerance to the kidney allograft despite transient mixed chimerism in non-human primates and humans. Evidence suggests that durable mixed chimerism may be required for tolerance induction of all types of allografts. In this study, we investigate whether co-infusion of amnion-derived multipotent progenitor (AMP) cells, which have unique immunomodulatory properties, can promote durable mixed allogeneic chimerism induction in a non-human primate model. Results suggest that intravenous infusion of high dose of 3rd party AMP cells did not lead to prolonged mixed chimerism, which was associated with the inability of AMP cells to traffic to or persist in the target organs. We will determine whether intrabone injection of AMP cells will allow these cells to exert their immunosuppressive activities to promote durable mixed chimerism induction.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. INTRODUCTION

Composite tissue reconstruction in the form of composite tissue allografts (CTA) represents a therapeutic option in the treatment of congenital abnormalities, oncologic surgery and traumatic injuries. Immunological rejection remains the major barrier for this therapy and its avoidance requires life-long potent immunosuppression, which itself has life-threatening adverse effects. Induction of tolerance to allografts is the ideal solution. Mixed chimerism induction via hematopoietic cell transplantation (HCT) has been shown to facilitate tolerance induction to kidney allografts in non-human primates and humans despite the transience of donor chimerism. However, evidence indicates that durable mixed chimerism may be required for tolerance induction to tissues or organs other than kidney. Amnion-derived multipotent progenitor (AMP) cells possess a unique immune phenotype and low immunogenicity and demonstrate immunosuppressive activities in vitro and in vivo in mouse models. In this project, we are investigating whether co-transplantation of AMP cells can promote the induction of durable mixed allogeneic chimerism in a highly clinically relevant non-human primate HCT model.

2. KEY WORDS

Mixed allogeneic chimerism, tolerance, amnion-derived multipotent progenitor (AMP) cells, non-human primate, composite tissue allograft

3. ACCOMPLISHMENTS

3a. Goal of this project

The major goal of this project is to develop an optimal regimen combining non-myeloablative conditioning, infusion of AMP cells and transient post-transplant immunosuppression for the induction of durable mixed allogeneic chimerism in cynomolgus monkeys so that tolerance will be applicable to CTA and all donor organ types.

We aim to extend and improve upon the 60-day chimerism achieved using the established non-myeloablative regimen consisting of low dose TBI, co-stimulatory blockade and 28 days of post-transplant immunosuppression with either cyclosporine or rapamycin. We will address the hypothesis that permanent, multilineage mixed chimerism will be achieved and this will be associated with robust donor-specific tolerance when AMP cells are given in the early post-BMT period.

3b. Accomplishments under these goals

3b-1. Specific Objective: Within this annual reporting period, we aimed to determine whether co-infusion of the highest dose of AMP cells would promote the induction of durable mixed allogeneic chimerism in non-human primates. To this end, we have performed bone marrow transplantation in two animals with a high dose of AMP cells and similar transplants in two animals that did not receive AMP cells. We have monitored the chimerism of these animals, performed in vitro assays to monitor their responses to donor alloantigens and look for the presence of injected AMP cells in tissues.

3b-2. Major Activities: To investigate the effects of the highest dose of AMP cells on the induction of durable mixed allogeneic chimerism in cynomolgus monkeys, the animals were conditioned with horse ATG, anti-CD40L, total body and thymic irradiation. In addition, rapamycin was administered for 30 days and tapered down for the following 21 days. On Day 0, the animal received donor bone marrow cells, with or without AMP cells (100 million/kg) i.v.

Following BMT, we monitored donor chimerism in peripheral blood by flow cytometric analysis twice a week and monitored the CMV levels in peripheral blood by PCR twice a week. Levels of rapamycin in blood were also monitored. In one animal receiving AMP cells, donor skin was grafted on Day 90. Anti-donor and anti-AMP antibodies in serum were analyzed by complement-dependent cytotoxicity assay or flow cytometry using anti-human IgM and anti-human IgG secondary antibodies. Mixed lymphocyte reactions were performed to determine the responses of host animals to donor alloantigens using peripheral blood mononuclear cells (PBMCs) as responder cells. The presence of AMP cells in tissues was determined by PCR. We have finished studies in 3 animals, including two animals receiving AMP cells and one control animal. The fourth animal, which is a control animal not receiving AMP cells, is currently being monitored at the time of submission of this report.

3b-3. Significant results and conclusions: Data from these animals lead to the following major findings:

- ***Co-transplantation of high dose of AMP cells did not prolong mixed chimerism***
  We monitored the donor chimerism in multiple hematopoietic cell lineages of animals receiving or not receiving AMP cells following transplantation. As shown in Figure 1, donor chimerism in lymphocytes, including T, B and NK cells, was much lower than that in monocytes and neutrophils in both animals receiving AMP cells (Figure 1A and 1B) and the control animal without AMP cells (Figure 1C). In the first (Figure 1A) and the second (Figure 1B) animals receiving AMP cells, donor chimerism in monocytes and neutrophils could be detected within the first week post-transplantation and increased over time, peaking on about Day 21 post-transplantation, while donor chimerism in lymphocytes remained low throughout the whole observation period. Donor chimerism in monocytes and neutrophils then started to decline gradually and completely disappeared on Day 41 (the first animal receiving AMP cells) and on Day 48 (the second animal receiving AMP cells) respectively, when donor chimerism in lymphocyte lineages in these two animals also disappeared. Reactivation of CMV, a common complication seen in our model, was detected in the first AMP animal on Day 17 post-transplant. Therapy with ganciclovir was initiated on Day 27 and foscarnet was added on Day 38 as CMV was still detected, although at a low level. The CMV was well controlled with the therapy afterward. Since CMV viremia was low during the loss of chimerism in this animal, it was unlikely that the reactivation of CMV was responsible for the loss of donor chimerism. Similarly, the correlation between loss of donor chimerism and reactivation of CMV in the second animal receiving AMP cells was also weak. Reactivation of CMV was detected on Days 17 and 31 with a low level of viremia, which was well controlled by anti-viral therapy. CMV viremia only lasted for a few days before coming under control (Figure 2B). Thus the complete loss of donor chimerism did not seem to be triggered by CMV reactivation in this animal.

  One animal underwent BMT with the same conditioning regimen and immunosuppression but no AMP cells to serve as a control for the two animals that have received AMP cells, in order to determine whether mixed chimerism was enhanced in the two animals receiving AMP cells. Following transplantation, donor chimerism in multiple lineages was detectable as usual within the first week. Donor chimerism in monocytes and neutrophils steadily increased over time and peaked by around Day 21, while chimerism in T, B and NK cells was detectable at much lower levels. In contrast to

the two animals that received AMP cells, whose donor chimerism started to decline after Day 21 following transplantation, the donor chimerism of this control animal plateaued in all lineages from Day 21 to Day 40, then began to decline (Figure 1C). CMV reactivation was first detected on Day 10 at a low level and viremia peaked from Day 22 to Day 30. Anti-viral therapy controlled the CMV and brought the viremia down to low levels in peripheral blood on Day 34 (Figure 2C). During the reactivation of CMV, mixed chimerism in all lineages did not show a significant decrease (Figure 1C). While donor chimerism remained stable, this animal demonstrated persistent pancytopenia requiring repeated blood transfusions. Its general condition deteriorated, which led to its death on Day 49. Within the last week before its death, donor chimerism declined, especially in monocytes and neutrophils, although it remained high at the time of death (Figure 1C). The cause of death of this animal is currently under investigation. Despite the unexpected death, the mixed chimerism was more persistent in this animal than in the two animals receiving AMP cells. We have now performed another control transplant, which is being monitored at the time of submission of this report. Taken together, results so far indicate that a high dose of AMP cells alone was not able to facilitate the induction of more durable mixed allogeneic chimerism in this model.

- ***Co-transplantation of a high dose of AMP cells did not facilitate induction of tolerance***
  In non-human primates and human patients, combined bone marrow and kidney transplantation resulted in tolerance to MHC-mismatched donor kidney allografts despite the transience of mixed chimerism. Although co-transplantation of a high dose of AMP cells did not lead to prolonged mixed chimerism, it remained possible that these cells could promote tolerance induction to donor alloantigens. The first animal receiving AMP cells was euthanized on Day 45 post-transplantation, after complete loss of donor chimerism. Mixed lymphocyte reactions using its frozen pre-transplant PBMCs and those harvested at the time of euthanasia as responders confirmed that tolerance to donor alloantigens had not been induced (Figure 3). A donor skin allograft, along with autologous and 3rd party skin allografts, was transplanted to the second AMP animal on Day 90 post-transplant, after donor chimerism had disappeared. However, all the skin grafts were lost due to technical issues. This animal was euthanized on Day 167 post-transplant. Mixed lymphocyte reaction was performed to determine the recipient T cell responses to donor alloantigens. Anti-donor antibodies in the serum were also assayed by complement-dependent cytotoxicity assay or flow cytometry using anti-human IgM and anti-human IgG secondary antibodies to assess humoral responses to the donor. As shown in Figure 4, mixed lymphocyte reaction using PBMCs harvested pre-transplantation and on Day 90 post-transplant demonstrated robust responses to donor stimulators, indicating that recipient T cells were not tolerized by the co-transplantation of AMP cells and donor bone marrow. In addition to T cell responses, anti-donor antibodies, mainly IgG, were detected on Day 91 post-transplant and later time points (Figure 5), which were able to mediate potent cytotoxicity against donor PBMCs in vitro (Figure 6). Thus, a humoral response to donor alloantigens had occurred in vivo. Collectively, these data demonstrate that co-transplantation of AMP cells did not lead to the induction of tolerance to donor alloantigens.

- ***Lack of persistence of AMP cells in vivo***
  We tested whether or not the human AMP cells were able to travel to and/or persist in recipient tissues. When we tested for the presence of human DNA, we detected no

evidence for AMP cells in bone marrow on Day 37 following infusion or in peripheral blood at multiple time points in the second animal receiving AMP cells (Figure 7), suggesting that the injected AMP cells were unable to travel to and/or persist in target tissues. In light of these data, we looked for factors that might lead to the destruction of AMP cells. We considered whether anti-AMP cell antibodies existed pre-transplantation or were induced at later time points post-transplant in the serum of the second animal receiving AMP cells. Indeed, anti-AMP cell antibodies, predominantly IgM, were detected in serum of this animal prior to transplantation. The levels of these anti-AMP cell antibodies remained constant throughout the observation period and no anti-AMP cell IgG antibodies were detected, indicating that the recipient animal was not primed by AMP cell-derived antigens (Figure 8 and 9). These results suggest that pre-existing anti-AMP cell antibodies might be a factor promoting rapid destruction of AMP cells and contributing to their lack of biological effect. Thus, our data suggest that approaches to delivering AMP cells to and enhancing their persistence in target tissues may be needed to facilitate their immunosuppressive effects in monkeys.

3C. Opportunities for training and professional development provided by this project

This project has provided training opportunities for Akaitz Dorronsoro-Gonzalez and Paula Alonso-Guallart, post-doctoral researchers. The training is through one-on-one work with me on this project.

3D. How were the results disseminated to communities of interest?

Nothing to report.

3E. What do you plan to do during the next reporting period to accomplish the goals?

Based on our data above, we will attempt to overcome two barriers in the next reporting period so that the AMP cells can exert their immunosuppressive effects. The first barrier is the pre-existing antibodies, which may mediate prompt removal of AMP cells following their infusion. The second is the inability of AMP cells to travel to and persist in target tissue. To overcome the first barrier, we will screen candidate animals to determine whether or not there are potential recipients with low levels of or no anti-AMP cell antibodies prior to transplant. To overcome the second barrier, we have obtained approval for intraosseus injection of AMP cells, as direct injection of AMP cells to the bone marrow may enhance their ability to survive, exert their immunosuppressive effects locally and thereby to promote induction of durable mixed chimerism. If intraosseus injection of AMP cells alone is not able to promote durable mixed chimerism, we will perform intraosseus injection of both AMP cells and ex-vivo expanded recipient polyclonal regulatory T cells.

Figures

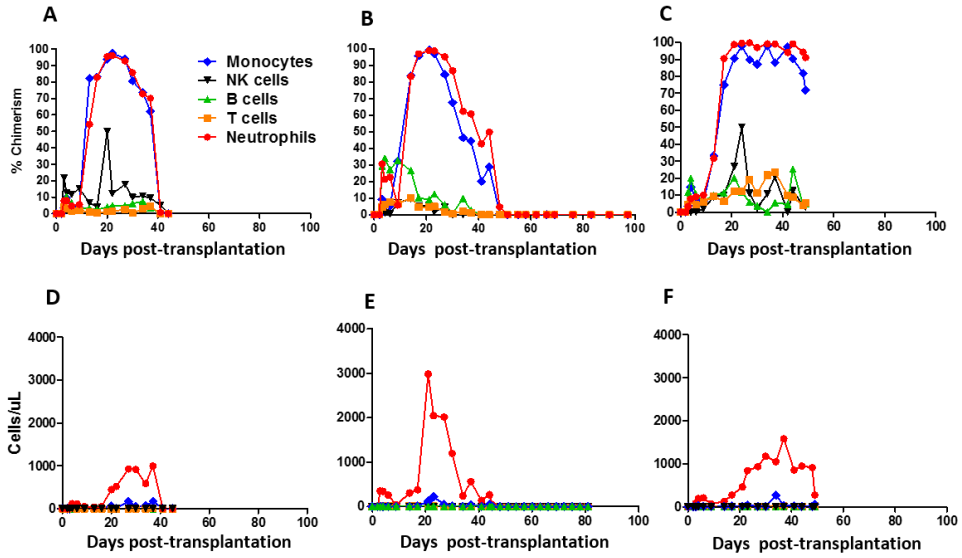


Figure 1. Donor chimerism expressed in percentages (upper panel, A-C) and absolute numbers (lower panel, D-F) in multiple lineages of animals receiving or not receiving AMP cells in peripheral blood following transplantation. (A, D) The first animal receiving AMP cells. (B, E) The second animal receiving AMP cells. (C, F) The control animal not receiving AMP cells.

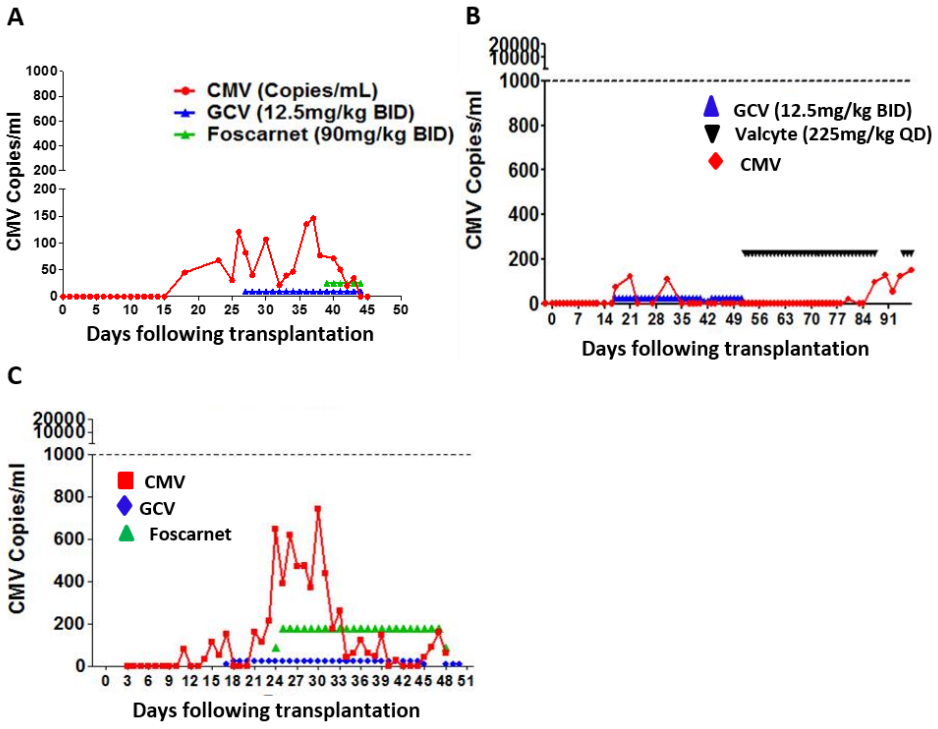


Figure 2. CMV levels in peripheral blood of animals receiving or not receiving AMP cells following transplantation. (A) The first animal receiving AMP cells. (B) The second animal receiving AMP cells. (C) The control animal without receiving AMP cells.

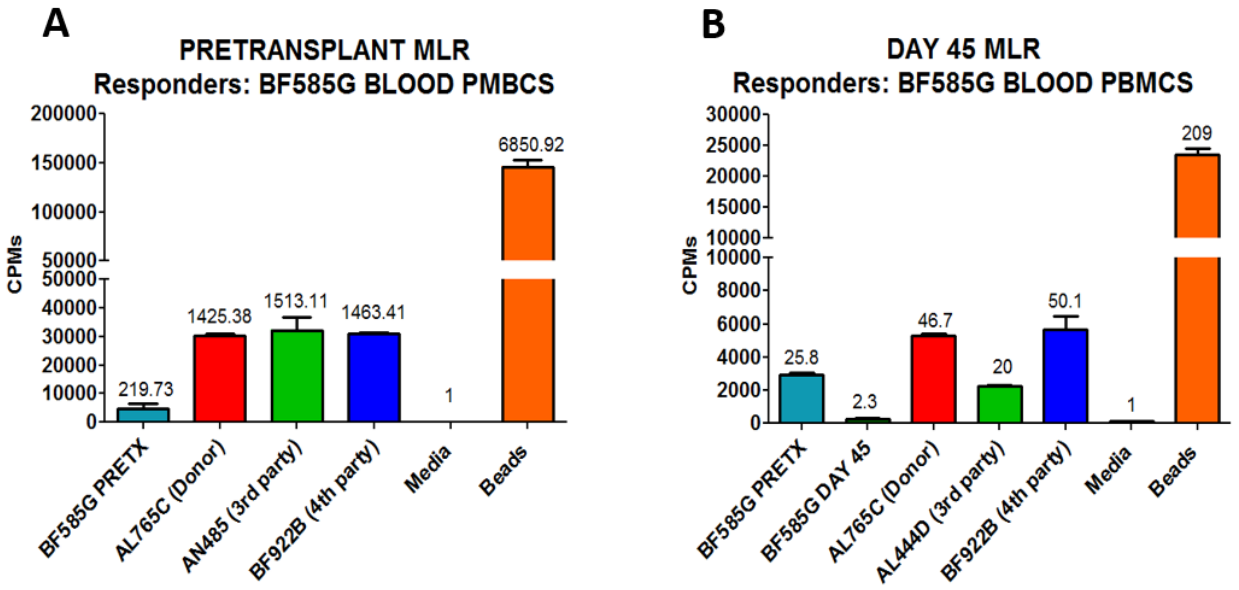


Figure 3. Responses of the first animal (BF585G) receiving AMP cells to donor alloantigens. Mixed lymphocyte reaction was performed prior to transplantation (A) and on Day 45 following transplantation (B) when the recipient was euthanized. PBMCs from the recipients were either unstimulated (Media), or stimulated with autologous, donor (AL765C), 3rd party (AN485), 4th party (BF922B) PBMCs and beads. Proliferation was determined by 3H incorporation. As shown, response of recipient PBMCs to donor stimulators was comparable to that to the 3rd party and 4th party stimulators, indicating that recipient T cells were not tolerant to the donor alloantigens. Numbers above each bar indicate stimulation index (cpm against stimulators/cpm with no stimulators)

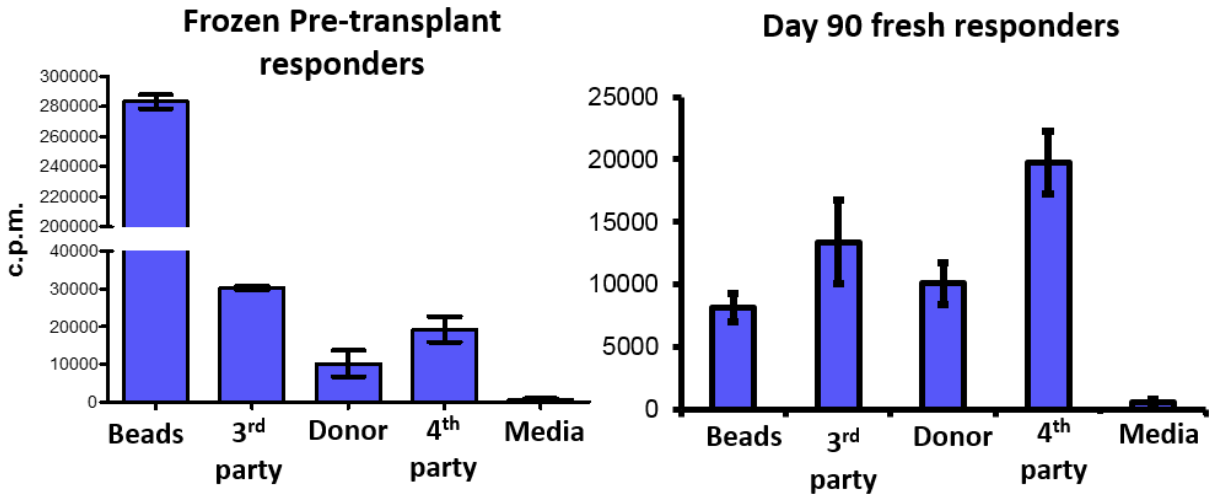


Figure 4. Responses to donor alloantigensof the second animal receiving AMP cells. Mixed lymphocyte reaction was performed using frozen pre-transplant PBMCs (A) and freshly isolated PBMCs (B) on Day 90 following transplantation as responders. PBMCs from the recipients were either unstimulated (Media), or stimulated with donor, 3rd party, 4th party PBMCs and beads. Proliferation was determined by ^{3}H incorporation. As shown, response of recipient PBMCs to donor stimulators was comparable to that to the 3rd party and 4th party stimulators, indicating that recipient T cells were not tolerant to the donor alloantigens.

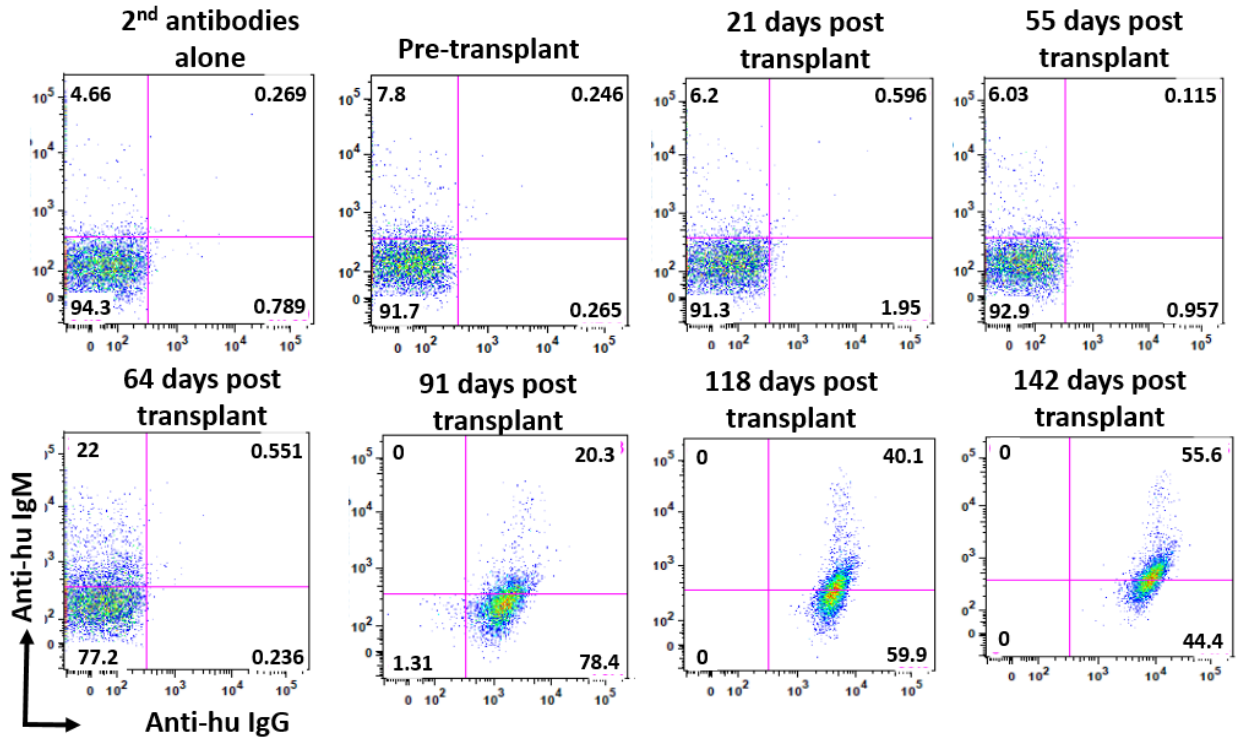


Figure 5. Anti-donor antibodies in the second animal receiving AMP cells. To determine if anti-donor antibodies were produced by the second animal receiving AMP cells, donor PBMCs were first incubated with serum from the recipient at different time points followed by incubation with anti-human IgM and anti-human IgG secondary antibodies and analyzed by flow cytometry. Donor PBMCs incubated with secondary antibodies but not serum served as control. No significant anti-donor antibodies were detected up to Day 55 post-transplant when the donor chimerism in peripheral blood was no longer detectable. On Day 64, significant amount of anti-donor IgM antibodies were detected and on Day 91 both anti-donor IgM and IgG antibodies were evident. These anti-donor antibodies were maintained afterwards. These data indicated that anti-donor humoral responses had been mounted to the donor and no tolerance of the recipient had been induced.

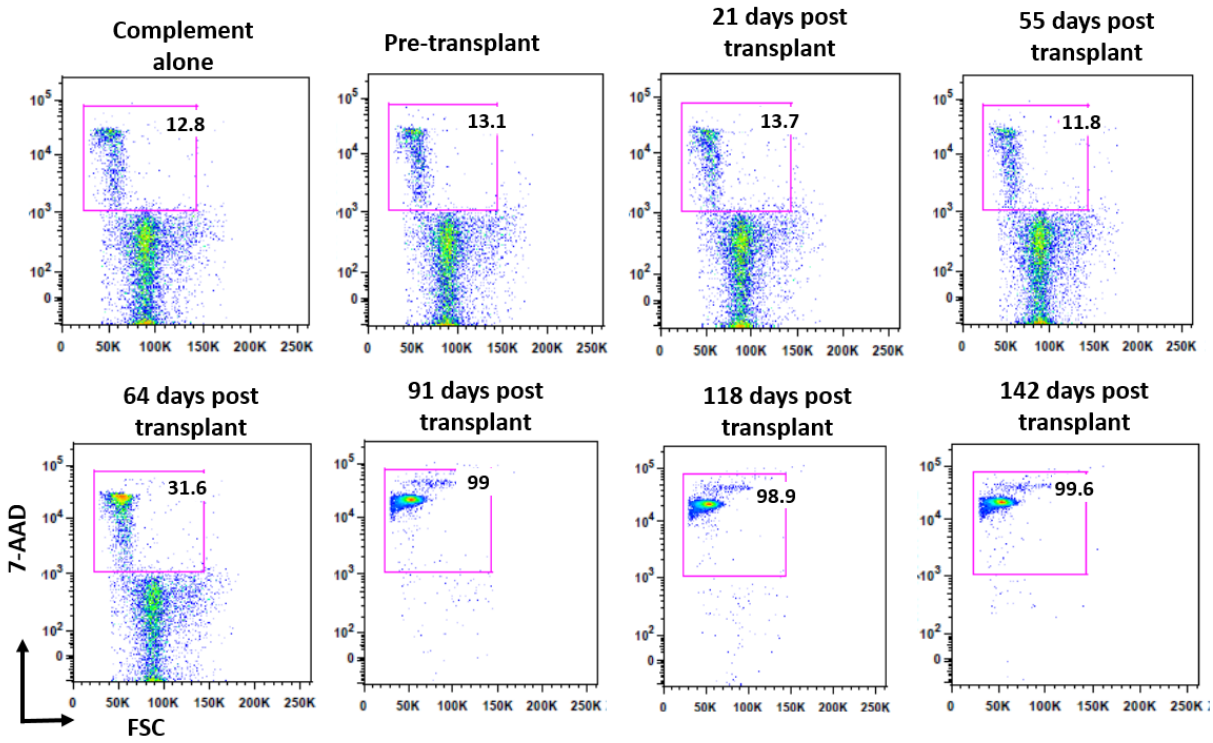


Figure 6. Anti-donor antibodies in the second animal receiving AMP cells determined by complement-dependent cytotoxicity assay. Donor PBMCs were first incubated with serum from the recipient at different time points followed by incubation with rabbit complement. 7-AAD was then added and analyzed by flow cytometry for 7AAD+ dead cells. Donor PBMCs incubated with complement without serum served as control. No significant anti-donor antibody-induced cell death was detected up to Day 55 post-transplant when the donor chimerism in peripheral blood was no longer detectable. On Day 64, significant amount of anti-donor antibody-induced cell death was detected and on Day 91 potent cell death was evident. Anti-donor antibody-induced cell death was maintained afterwards. Consistent with data in Figure 5, these data indicated that anti-donor humoral responses had been mounted to the donor and no tolerance of the recipient had been induced.

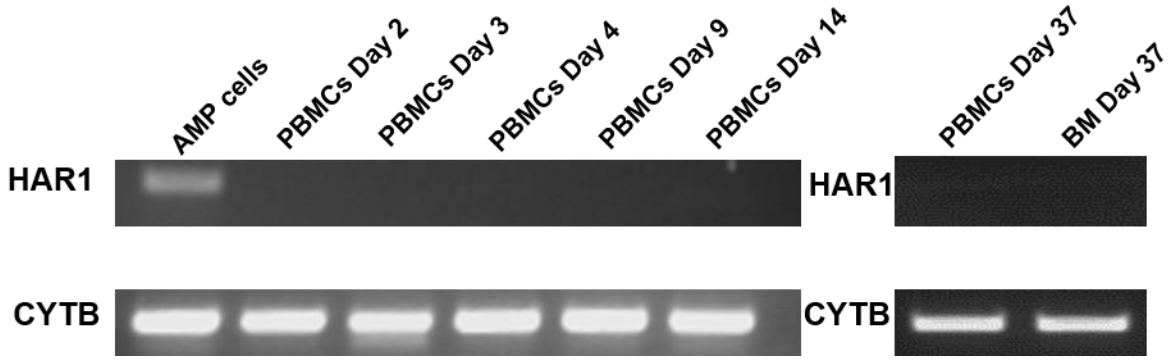


Figure 7. Absence of AMP cells in peripheral blood and bone marrow. PCR was performed to detect AMP cells in peripheral blood (PBMCs) at multiple time points and in bone marrow (BM) on Day 37 in the 2nd animal receiving AMP cells. AMP cells were not detected in these tissues any time point. HAR1: human accelerated region 1. CYTB: cytochrome b (present in the mitochondrial genome). CYTB is used as a housekeeping gene. Primers for HAR1 were specific for human sequence. This PCR was able to detect DNA from a minimum of 4000 human cells. 60,000 to 180,000 PBMCs and 780,000 bone marrow cells were used for PCR.

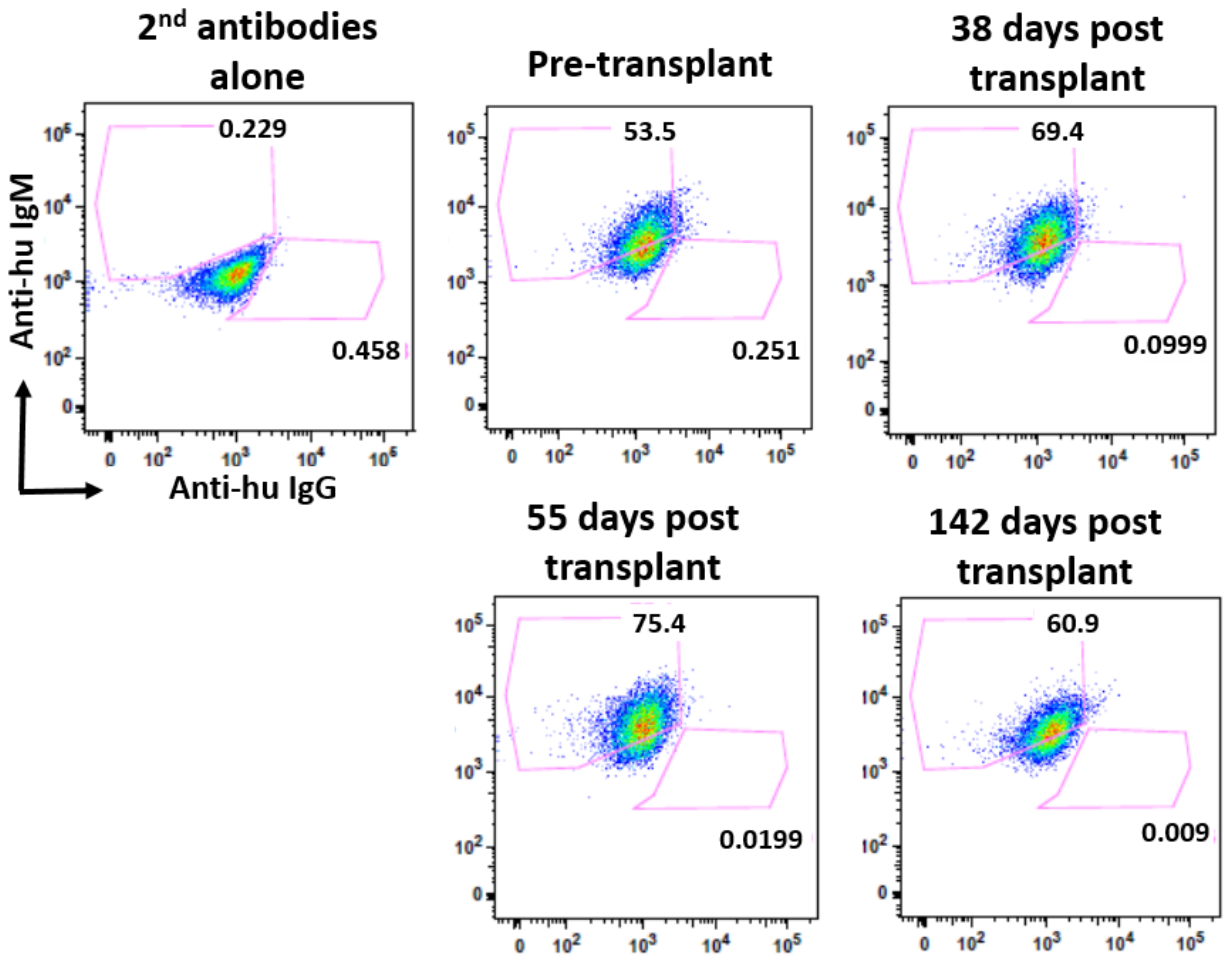


Figure 8. Anti-AMP cell antibodies in the second animal receiving AMP cells. To determine if anti-AMP cell antibodies were produced by the second animal receiving AMP cells, AMP cells were first incubated with serum from the recipient at different time points followed by incubation with anti-human IgM and anti-human IgG secondary antibodies and analyzed by flow cytometry. Donor PBMCs incubated with secondary antibodies but not serum served as control. Anti-AMP cell antibodies, predominantly IgM were detected prior to transplant and at different time points post-transplant. These data demonstrated the presence of pre-existing anti-AMP cell antibodies and suggested that the recipient was not primed by the AMP cell-derived antigens.

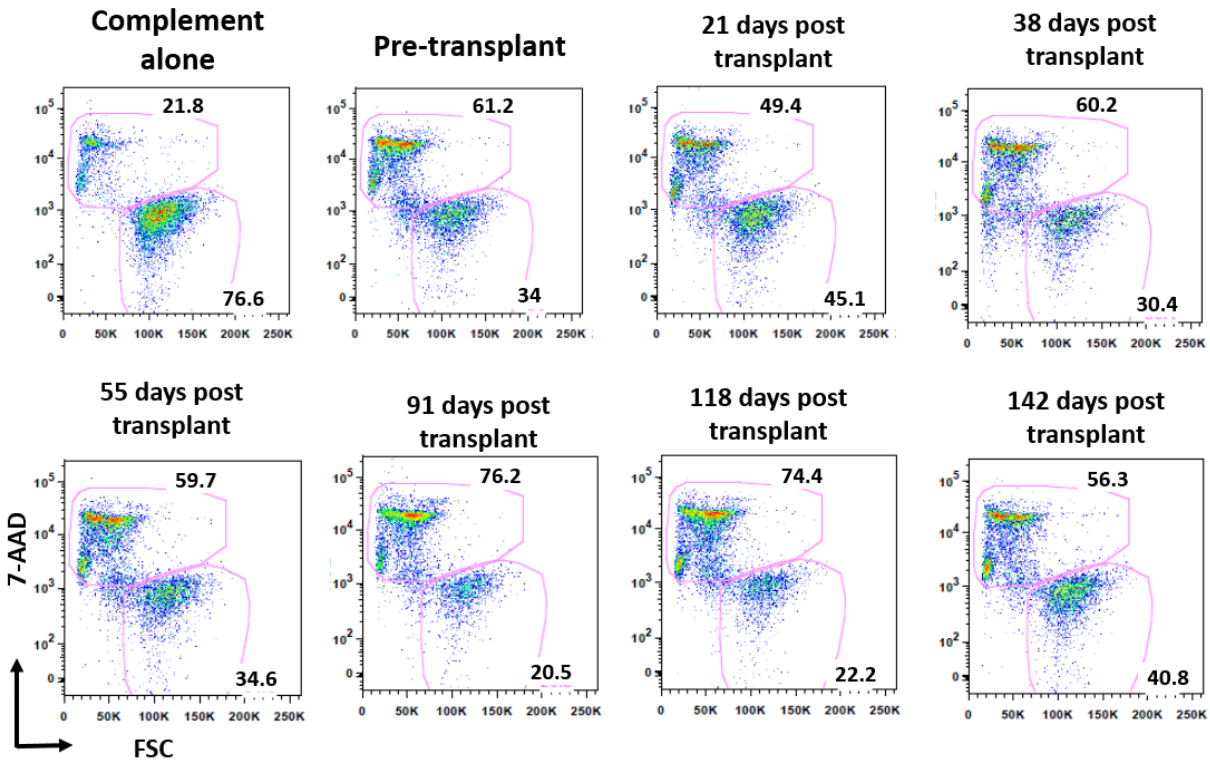


Figure 9. Anti-AMP cell antibodies in the second animal receiving AMP cells detected by complement-dependent cytotoxicity assay. AMP cells were first incubated with serum from the recipient at different time points followed by incubation with rabbit complement. 7-AAD was added and 7-AAD+ dead cells were analyzed by flow cytometry. AMP cells incubated with complement without serum served as control. Consistent with data in Figure 8, anti-AMP cell antibodies were detected prior to transplant and at different time points post-transplant. These data demonstrated pre-existing cytotoxic anti-AMP cell antibodies and suggested that the recipient was not primed by the AMP cell-derived antigens.

4. IMPACT

4A. What was the impact on the development of the principle discipline(s) of the project?

Important information on the barriers to testing this human stem cell type in this pre-clinical model was being obtained.

4B. What was the impact on other discipline(s)?

The demonstration of monkey anti-human natural antibodies has implications for the testing of other human cell types in monkey models.

4C. What was the impact on technology transfer?

We have shared our results with Noveome Biotherapeutics (formerly Stemnion Inc.), who have provided the AMP cells for the study.

4D. What was the impact on society beyond science and technology?

None so far.

5. CHANGES/PROBLEMS

5A. Changes in approach and reasons for change

There will be two major changes in approach. As we have found that monkeys have anti-AMP cell natural antibodies, which may lead to rapid destruction of the AMP cells, we will screen the candidate animals to determine whether or not there are potential recipients with low levels of or no anti-AMP cell antibodies prior to this transplant. The inability of AMP cells to travel to and persist in target tissues may have hampered their immunosuppressive effects. Instead of intravenous injection, we will perform intraosseus injection of AMP cells so that they can directly exert their immunosuppressive effects in bone marrow.

5B. Actual or anticipated problems or delays and actions or plans to resolve them

We have encountered technical issues in skin grafting. We used frozen skin for grafting, which increases the technical difficulty. We will consult a plastic surgeon to improve the skin grafting protocol.

5C. Changes that had a significant impact on expenditures

Nothing to report

5D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

We have modified the protocol to allow for intraosseous injection of AMP cells and stem cells. We obtained approval from IACUC at Columbia University on July 26, 2016 for this modification in animal protocol AC-AAAN2750 and subsequently approval from the Animal Care and Use Review Office on Aug 24, 2016 (USAMRMC Proposal Number MR141045). In our initial safety studies, we discovered that AMP cells were being trapped in the pulmonary capillaries after intravenous infusion, preventing their migration to the bone marrow. We have now demonstrated that primates contain natural anti-human IgM antibodies that may eliminate AMP cells in circulation. We hypothesize that direct delivery of AMP cells to the bone will decrease the loss of AMP cells in the pulmonary vasculature and decrease destruction due to preformed antibodies. We expect intraosseus injection to enhance their biologic effect on stem cell engraftment. A mixture of AMP cells and donor stem cells suspended in a collagen gel matrix are injected into the tibial tuberosity using a 16-guage needle after inducing general anesthesia and sterile preparation of the site. A pressure bandage is applied to prevent bleeding. Post-operative analgesia is performed per protocol.

6. PRODUCTS:

6A. Publications, conference papers, and presentations

Abstract

P Guallart, J Zitsman, H Sondermeijer, S Chaudry, J Weiner, A Griesemer, R Tokarz, M Pereira, S Hammer, M Sykes and R Duran-Struuck. Non-human primate model for CMV viremia: immunologic impact and treatment approaches. FOCIS 2016, Boston, MA

6B. Journal publications

Manuscript in Revision

R Duran-Struuck, H Sondermeijer, L Bühler, P Guallart, J Zitsman, Y Kato, A Wu, A McMurchy, D Woodland, A Griesemer, M Martinez, S Boskovic, T Kawai, AB Cosimi, C Wuu, A Slate, M Mapara, S Baker, R Tokarz, V D' Agati, S Hammer, M Pereira, WI Lipkin, T Wekerle, M Levings, M Sykes. Effect of ex vivo expanded recipient regulatory T cells on hematopoietic chimerism and kidney allograft tolerance across MHC barriers in cynomolgus macaques. Manuscript in Revision (Submitted).

6C. Books or other non-periodical, one-time publications

Nothing to report

6D. Other publications, conference papers, and presentations

Nothing to report

6E. Website(s) or other Internet site(s)

Nothing to report

6F. Technologies or techniques

Nothing to report

6G. Inventions, patent applications, and/or licenses

Nothing to report

6H. Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7A. What individuals have worked on the project?

Name:	Megan Sykes (No change)
Project Role:	Principal Investigator
Researcher Identifier (UNI of Columbia University): ms3976	
Nearest person month worked:	0.5
Contribution to Project: Leadership	
Name:	Adam Griesemer (No change)
Project Role:	Co-Investigator
Researcher Identifier (UNI of Columbia University): adg2101	
Nearest person month worked:	1
Contribution to Project: Leadership, training, surgeries, analysis	
Name:	Paula Alonso Guallart (No change)
Project Role:	Post-doctoral researcher
Researcher Identifier (UNI of Columbia University): pa2396	
Nearest person month worked:	2
Contribution to Project: execution of aims, analysis	
Name:	Dilrukshi Ekanayake-Alper (increased effort)
Project Role:	Veterinarian researcher
Researcher Identifier (UNI of Columbia University): dke2107	
Nearest person month worked:	2
Contribution to Project: execution of aims, animal care, analysis	

Name:	Akaitz Dorronsoro Gonzalez (departed)
Project Role:	Post-doctoral researcher
Researcher Identifier (UNI of Columbia University): ad3328	
Nearest person month worked:	9
Contribution to Project: execution of aims, analysis	
Name:	Hao Wei Li (No change)
Project Role:	Co-Investigator
Researcher Identifier (UNI of Columbia University): hl2591	
Nearest person month worked:	2
Contribution to Project: execution of aims, training, analysis	

Others with nominal effort:
Chengshie Wuu, radiation physicist <1 month effort in supervising radiations; Makenzie Danton and Sigal Kofman, technicians <1 month effort covering animal care and procedures.

7B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Megan Sykes, MD

New funding:

P01 AI045897 (PI: Sykes/Sachs) 08/19/16 – 07/31/17 1.44 calendar months
NIH/NIAID $1,937,112
A Tolerance Approach to Xenotransplantation; Proj 3: Tolerance of Adaptive and Innate Human Anti-Pig Immune Responses in Humanized Mice (Proj. Leader Sykes); Admin Core leader

Stemnion research agreement (PI: Sykes) 01/20/15-12/31/17 0.12 calendar months
Stemnion, Inc. $53,078
Facilitating mixed allogeneic chimerism induction by co-transplantation of Amnion-derived Multipotent Progenitor Cells (AMP cells) in humanized mice with established immune system

P01HL018646 (PI: Madsen) 11/14/14 – 10/31/19 0.48 calendar months
NIH/NHLBI $64,078
New Approaches to Cardiothoracic Tolerance Induction (Co I: Sykes)

R01 DK103585-01A1 (PI: Sykes) 04/01/15 – 03/31/19 0.96 calendar months
NIH/NIDDK/OD $217,854 + $67,022 diversity supplement
Immune response to iPSC-derived beta cells in Type 1 diabetes

R01 OD017949 (Sykes) 07/01/15 – 03/31/20 0.96 calendar months
NIH/OD $489,997
Robust allograft tolerance in non-human primates

Spons. Proj. Agreement (PI: Sykes/Sachs) 08/01/15 – 07/31/18 1.2 calendar months
United Therapeutics/Lung Biotechnology $1,014,197
Collaboration on Xenograft Lung Tolerance

Spons. Research Agreement (PI: Sykes) 02/18/16-01/17/18 0.12 calendar months
CELLDEX Therapeutics $63,334
Combined use of Flt3L and rapamycin to promote durable mixed allogeneic chimersim

UM1 AI109565 (PI: Nepom) 02/01/16-01/31/17 0.48 calendar months
NIH/NIAID $150,000
ITN Study: Tracking donor-reactive Treg TCR as marker of tolerance

R56AI122332-01A1 (PI: Sykes) 08/19/16-07/31/17 0.36 calendar months
NIH/NIAID $200,000
Immune response to combined liver and bone marrow transplant for tolerance in NHP

Expired funding:
NIH/NIAID R01AI084903
NIH/NIAMS R21AR064473
NIH/NIAMS P30AR044535
Frederick Banting Foundation Award
Helmsley Foundation Award
NIH/NIAID/ITN UM1AI09565

Adam Griesemer, MD
New funding:
P01 AI045897 (PI: Sykes/Sachs) 08/19/16 – 07/31/17 1.2 calendar months
NIH/NIAID $1,937,112
A Tolerance Approach to Xenotransplantation; Proj 2: (Co I: Griesemer)

R01 OD017949 (Sykes) 07/01/15 – 03/31/20 1.2 calendar months
NIH/OD $489,997
Robust allograft tolerance in non-human primates (Co I: Griesemer)

Spons. Proj. Agreement (PI: Sykes/Sachs) 08/01/15 – 07/31/18 1.8 calendar months
United Therapeutics/Lung Biotechnology $1,014,197
Collaboration on Xenograft Lung Tolerance (Project Leader: Griesemer)

R56AI122332-01A1 (PI: Sykes) 08/19/16-07/31/17 0.36 calendar months
NIH/NIAID $200,000
Immune response to combined liver and bone marrow transplant for tolerance in NHP (Co I: Griesemer)

Expired funding:
Louis V. Gerstner scholarship/Career Award

7C. What other organizations were involved as partners?
Noveome Biotherapeutics, Inc. (f.k.a. Stemnion, Inc.)
100 Technology Drive
Suite 200
Pittsburgh, PA 15219
In kind support: provision of AMP cells

8. SPECIAL REPORTING REQUIREMENTS
8A. Collaborative awards

Nothing to report

8B. Quad charts

Attached

8C. Appendices

None

Establishment of donor chimerism in non-human primates using allogeneic bone marrow with AMP cell co-infusion.

Insert ERMS/Log Number and Task Title Here
Insert Award Number Here

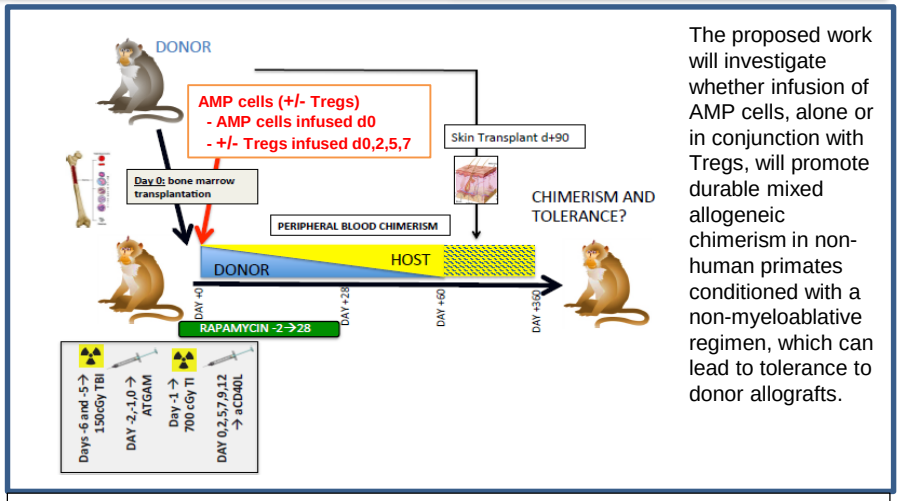
PI: Megan Sykes, MD **Org:** Columbia University **Award Amount:** $1,200,000 total cost dollars

Study/Product Aim(s)

- Determine whether co-transplantation of amnion-derived multipotent progenitor (AMP) cells, alone or with regulatory T cells (Tregs), can promote durable mixed chimerism and induce tolerance
- Modification of bone marrow transplantation protocol for clinical cadaveric organ transplantation

Approach

AMP cells, alone or together with Tregs, will be co-infused with allogeneic bone marrow cells to non-human primates conditioned by a non-myeloablative regimen to induce mixed hematopoietic chimerism. Donor chimerism will be followed and tolerance will be determined by skin graft.



The proposed work will investigate whether infusion of AMP cells, alone or in conjunction with Tregs, will promote durable mixed allogeneic chimerism in non-human primates conditioned with a non-myeloablative regimen, which can lead to tolerance to donor allografts.

Accomplishment: Demonstrated that expanded recipient Tregs can enhance and prolong chimerism and prolong donor skin graft survival

Timeline and Direct Cost Dollars

Activities CY	15	16	17
Determine effects of AMP cells alone	→	→	
Determine effects of AMP cells with Tregs		→	→
Modification of transplantation protocol for clinical cadaveric organ transplantation			→
Estimated Budget (direct $)	$64,000	$311,000	$375,000

Updated: (8/30/16)

Goals/Milestones

CY15 Goal – Determine the effects of AMP cells on mixed chimerism induction and tolerance

- ❑ Start studies with infusion of the highest dose of AMP cells

CY16 Goals – Determine dose effects of AMP cells or synergistic effects with Tregs

- ❑ Complete studies with infusion of the highest dose of AMP cells
- ❑ Start studies with infusion of AMP cells and Tregs

CY17 Goal – Modification of transplantation protocol for clinical cadaveric organ transplantation

- ❑ Complete studies with infusion of AMP cells and Tregs
- ❑ Modify transplantation protocol for clinical cadaveric organ transplantation based on results CY15 and CY16

Budget Expenditure to Date

Projected Expenditure: $600,000 total cost (whole year)
Actual Expenditure: $412,168 total cost (actual + encumbered)